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The Effects of Temperature on the Fatty Acid and Phospholipid Composition of Four Obligately Psychrophilic *Vibrio Spp.*

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Abstract. The free fatty acid and phospholipid composition of 4 psychrophilic marine *Vibrio* spp. have been determined in chemostat culture with glucose as the limiting substrate over a temperature range 0–20° C. All the isolates show maximum glucose and lactose uptake at 0° C and this correlates with maximum cell yield. None of the isolates contain fatty acids with a chain length exceeding 17 carbon atoms. *Vibrio* AF-1 and *Vibrio* AM-1 respond to decreased growth temperatures by synthesizing increased proportions of unsaturated fatty acids (C15:1, C16:1 and C17:1) whereas in *Vibrio* BM-2 the fatty acids undergo chain length shortening. The fourth isolate (*Vibrio* BM-4) contains high levels (60 %) of hexadecenoic acid at all growth temperatures and the fatty acid composition changes little with decreasing temperature. The principal phospholipid components of the four psychrophilic vibrios were phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. Lyso-phosphatidylethanolamine and 2 unknown phospholipids were additionally found in *Vibrio* AF-1. The most profound effect of temperature on the phospholipid composition of these organisms was the marked increase in the total quantities synthesized at 0° C. At 15° C phosphatidylglycerol accumulated in the isolates as diphosphatidylglycerol levels decreased. Additionally in *Vibrio* BM-2 and *Vibro* BM-4 phosphatidylserine accumulates as phosphatidylethanolamine biosynthesis was similarly impaired. The observed changes in fatty acid and phospholipid composition in these organisms at 0° C may explain how solute transport is maintained at low temperature.

Key words: Psychrophiles – *Vibrio* – Fatty acids – Phospholipids – Cell yield – Substrate uptake.

Considerable interest has been shown in the lipid composition of microorganisms in relation to their response to growth at low or sub-optimum growth temperatures (Kates and Baxter, 1962; Farrell and Rose, 1967; Brown and Rose, 1969; Cullen et al., 1971). This fascination stems from the fact that lipids form the bulk of the cytoplasmic membrane and hence any alterations in the physical and chemical composition of the membrane lipids may also result in altered membrane function. The most noticeable feature observed in microoorganisms upon decreasing the growth temperature is the rapid synthesis of increased proportions of unsaturated fatty acids, particularly hexadecenoic and octadecenoic acids at the expense of their respective saturated acids. Several workers (Gaughran, 1947; Farrell and Rose, 1967) have argued that the physiological significance of increased unsaturated fatty acid levels is to decrease the melting point of the membrane lipids thereby maintaining their integrity and function. Direct evidence for this hypothesis is still lacking but data from substrate uptake studies by psychrophilic and mesophilic yeasts and bacteria provide indirect support for the theory. Baxter and Gibbons (1962) first demonstrated in several *Candida* spp. that substrate uptake was largely independent of temperature. Similarly, Russell (1971) showed that in the psychrophilic bacterium *Micrococcus cryophilus* lysine uptake occurred at the same rate at 0°C as at 20°C whilst in a more recent study Herbert and Bell (1977) demonstrated that in the psychrophilic *Vibrio* AF-1 maximum uptake of glucose

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Abbreviations: Phosphatidylserine, PS; phosphatidylethanolamine, PE; phosphatidylglycerol, PG; diphosphatidylglycerol, DPG; lysophosphatidylethanolamine, lyso PE

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and lactose occurred at 0° C and decreased with increasing temperature. Several authorities (Ingraham and Bailey, 1959; Rose and Evison, 1965; Morita and Buck, 1974) consider that the failure of mesophilic organisms to grow at low temperatures (< 5° C) is a function of their inability to maintain mobility of their membrane lipids with the result that substrate uptake is severely inhibited. Work by Hunter and Rose (1972) using the mesophilic yeast *Saccharomyces cerevisiae* failed to detect significant changes either in fatty acid or degree of unsaturation when grown at 15° C and 30° C whereas in psychrophilic yeasts *Leucosporidium frigidum*, *Leucosporidium gelidum* and *Leucosporidium nivalis* grown at −1° C, 90% of the total fatty acids were unsaturated with linolenic and linoleic predominating (Watson et al., 1976). In bacteria the change in fatty acid composition in response to decreased growth temperature is less clear. Kates and Hagen (1964) examined mesophilic and psychrophilic *Serratia* spp. and concluded that the psychrophilic species produced increased levels of unsaturated fatty acids at low temperatures compared with mesophiles. In *M. cryophilus* the response is somewhat different and involves shortening of the fatty acid chain length (Russell, 1971) whilst Brown and Minnikin (1973) observed no significant changes in the fatty acid composition of several marine psychrophilic pseudomonads when grown at different temperatures. In this study we have investigated the effect of growth temperature on the fatty acid composition of 4 psychrophilic *Vibrio* spp. isolated from Antarctic and North Sea sediments. To avoid complications due to changes in lipid content as a function of growth rate the cultures were grown in a chemostat at a fixed dilution rate.

Materials and Methods

Organisms. The 4 psychrophilic *Vibrio* spp. were isolated from marine sediments. Isolates AF-1 and AM-1 were obtained from Signy Island, South Orkney Islands, Antarctica and BM-2 and BM-4 from the North Sea. All were identified as *Vibrio* spp. using conventional morphological and biochemical tests (Cowan and Steel, 1965).

Growth conditions. The organisms were grown up in a single stage 1 l chemostat as described by Baker (1968) under glucose limitation. Growth conditions and media used were as described by Herbert and Bell (1977).

Extraction and Analysis of Phospholipids. The phospholipids from freeze dried bacteria (20 − 30 mg) were initially extracted by stirring with 5 ml chloroform-methanol (2:1 vol/vol) for 12 h at 4° C. The cell pellet was extracted with a further 5 ml chloroform/methanol and the two supernatants combined and evaporated to dryness by a stream of high purity nitrogen gas. The extracts were stored in a deep-freeze until required for phospholipid analysis.

Estimation, Separation and Identification of Phospholipids. The extracted phospholipids were separated by two dimensional thin layer chromatography on 0.2 mm thick layers of activated Silica-gel G (Merck, Darmstadt) impregnated with 0.2% w/v sodium acetate using chloroform/methanol/water (65:25:4% v/v) in the first dimension and chloroform/acetic acid/methanol/water (80:18:12:5% v/v) in the second dimension. Individual phospholipids were identified on the basis of R_f values in several solvent systems (chloroform/methanol/water 65:25:4% v/v; chloroform/acetic acid/methanol/water, 80:18:12:5; chloroform/methanol/7 N ammonia, 60:35:5; chloroform/methanol/7 N ammonia, 35:60:5), by reaction with specific detecting reagents (ninhydrin, molybdate, periodate Schiff reagents) and by co-chromatography with authentic phospholipids obtained from Sigma Chemicals Ltd.

For quantitative estimations the lipids were revealed by spraying the dried chromatograms with a saturated solution of potassium dichromate in conc. H_2SO_4 followed by charring at 200° C for 20 min. The chromatograms were then photographed and positives made which were then scanned by a double beam recording microdensitometer (Joyce, Loebl and Co. Ltd.). Quantitative determinations of each phospholipid component were then determined by integrating the peak area and comparing with standard curves prepared using authentic phospholipids. Standard curves were prepared using 2, 5, 7.5, 10 and 15 µg quantities of individual phospholipids obtained from Sigma Chemicals Ltd. by the method described above.

Extraction and Methylation of Free Fatty Acids. Duplicate samples of freeze dried cells (20 − 30 mg) to one of which was added myristic acid as internal standard were extracted with 10 ml chloroform : methanol (2:1 v/v) by shaking in a wrist action shaker for 1 h followed by overnight incubation at 4° C. The supernatant was decanted, evaporated to dryness and redissolved in petroleum ether (40 − 60° C B.P.) followed by a further drying stage.

Methyl esters were prepared by refluxing the extracts with 4 ml of methanol/6 M HCl (14:1 v/v) plus 0.5 ml benzene for 2 h at 90° C. The methyl esters were extracted with petroleum ether (40 − 60° C B.P.) and taken down to dryness using high purity nitrogen. The methyl esters were stored at −23° C until required.

Gas Liquid Chromatography. Long chain fatty acid methyl esters were analysed by gas chromatography using a Pye 104 gas chromatograph fitted with a glass column (200 × 0.4 cm) packed with 10% (w/w) polyethyleneglycol adipate on Chromosorb W (100 − 200 mesh) with N_2 as carrier gas at a temperature of 175° C. The identity of individual fatty acid methyl esters was determined by comparison of retention times and equivalent chain lengths (Ackmann, 1963) on the above column as well as a 200 × 0.4 cm glass column packed with 15% (w/w) Apiezon L on AWS Chromosorb W (80 − 100 mesh) at a temperature of 190° C with those of standard mixtures of straight chain and unsaturated methyl esters obtained from Sigma Chemicals. The identity of unsaturated fatty acid methyl esters were confirmed by their disappearance after hydrogenation (Kates, 1972). Cyclopropane peaks do not shift position under these conditions. The relative proportions of the fatty acids were calculated from the peak areas estimated from peak height × peak width at half peak height.

Uptake of ^{14}C-Labelled Substrates. Uptake of ^{14}C-glucose and ^{14}C-lactose at different growth temperatures were determined according to the method of Herbert and Bell (1977).

Results

Data presented in Table 1 show the principal taxonomic characteristics of the 4 psychrophilic bacteria studied in this investigation. All are asporogenous, oxidase and catalase positive Gram-negative rods which ferment glucose without gas production under anaerobic conditions. They are all arginine negative

Table 1. Taxonomic characteristics of the 4 psychrophilic isolates

Taxonomic character	Isolate No.			
	AF-1	AM-1	BM-2	BM-4
Morphology	rod	rod	rod	rod
Gram stain	−	−	−	−
Motility	+	+	+	+
Flagella	single polar	single polar	single polar	single polar
Oxidase	+	+	+	+
Catalase	+	+	+	+
Hugh & Liefsons O/F test	F	F	F	F
Arginine dihydrolase	−	−	−	−
0/129 sensitivity	+	+	+	+
Optimum growth temperature	15°C	6°C	4°C	8°C
Maximum growth temperature	20°C	15°C	15°C	18°C
Gelatin liquefaction	+	+	+	+
Citrate utilization	+	+	+	+

and sensitive to the vibriostatic reagent 0/129. According to the taxonomic scheme of Shewan et al. (1960) they have been classified as *Vibrio* spp. Optimum growth temperatures range from 4°C (BM-2) to 15°C (AF-1) whilst none of the isolates grew at temperatures exceeding 20°C with glucose as carbon source confirming that these are psychrophilic bacteria according to the definition of Morita (1975).

The results of lactose and glucose uptake experiments for *Vibrio* AF-1 grown in continuous culture at different temperatures are given in Fig. 1. These data show that maximum uptake of both ^{14}C-glucose and ^{14}C-lactose occur at 0°C and decrease with increasing growth temperature. Whilst maximum sugar uptake does not occur at 15°C, the optimum growth temperature for *Vibrio* AF-1 (Table 1), it does correlate with maximum cell yield, cell size and minimum oxygen consumption (Herbert and Bell, 1977). Similar observations have been made for the other *Vibrio* spp. studied. To facilitate substrate uptake at low temperatures the lipid solidification theory proposed by Byrne and Chapman (1964) postulates that the membrane lipids must remain mobile at low temperatures. Detailed investigations of the fatty acid and phospholipid composition of the 4 psychrophilic *Vibrio* spp. and how they respond to decreased growth temperatures are given in Table 2 and 3.

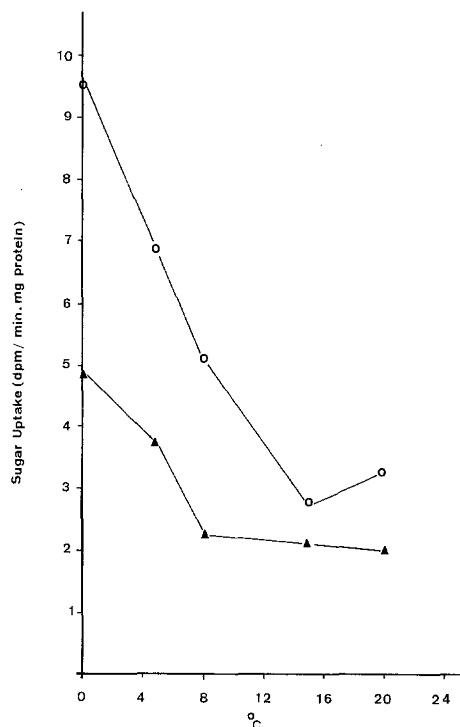

Fig. 1. Effect of growth temperature on the uptake of ^{14}C-glucose and ^{14}C-lactose by *Vibrio* AF-1 grown under glucose limitation. ○——○ uptake of ^{14}C-glucose; ▲——▲ uptake of ^{14}C-lactose

Data presented in Table 2 show that none of the isolates contain fatty acids with chain lengths greater than 17 carbon atoms. Fatty acid profiles of *Vibrio* AF-1 show the predominance of C14, C15 and C16 saturated and unsaturated acids. Decreasing the growth temperature from 15–0°C involved the increased synthesis of unsaturated components, notably pentadecenoic and hexadecenoic at the expense of saturated fatty acids. In addition, significant quantities of heptadecenoic acid are synthesized only at 0°C. The fatty acid pattern of *Vibrio* AM-1 shows a similar response to that observed with *Vibrio* AF-1 in respect to decreasing the growth temperature from 15–0°C. In this organism the dominant fatty acids are hexadecenoic and heptadecenoic acids and comprise approximately 60% of the total acids at 0°C and 8°C whereas at 15°C they

Table 2. Effect of growth temperature on the fatty acid composition of the 4 *Vibrio* spp. grown under glucose limitation

Fatty acid[a]	*Vibrio* AF-1 growth temperature			*Vibrio* AM-1 growth temperature			*Vibrio* BM-2 growth temperature			*Vibrio* BM-4 growth temperatu		
	0°C	8°C	15°C	0°C	8°C	15°C	0°C	8°C	15°C	0°C	8°C	15°C
8:0	0	0	0	0.7	0.5	9.6	7.9	6.3	4.6	0.5	0.7	0.7
9:0	0.9	0.6	0	0	0	0	0	0	0	0	0	0
9:1	0.4	0.6	0	0	0	0	11.4	6.6	5.4	0	0	0
10:0	0.4	6.3	9.8	2.0	2.4	8.9	0	0	0	1.5	0.4	0.6
11:0	0	4.0	4.7	0.7	1.7	0.4	0	0.6	0.7	1.2	1.8	2.4
12:0	0.4	4.2	0.5	0	0	8.0	0	6.4	9.0	0.4	0.9	0.8
12:1	0	0	1.8	8.2	10.7	1.0	13.7	1.0	0	0.4	0.4	0
13:0	0	1.3	2.6	0	0	0	0	0	0	0	0	0
13:1	1.6	1.0	1.8	0	0	0	0	0	0	1.3	0	0
14:0	7.1	22.6	8.1	1.2	1.7	21.8	0	1.0	1.2	1.5	11.3	15.9
14:1	8.5	7.6	15.0	5.5	0	0	20.6	7.5	12.0	4.2	0	0
15:0	19.4	4.7	8.6	0	0	0	0.7	0.9	1.1	8.3	3.6	3.0
15:1	26.7	27.0	13.5	8.2	1.0	18.2	1.5	2.5	2.9	9.5	6.8	0
15:Δ	0	11.1	6.4	0	0	0	0	0	0	0	0	0
16:0	10.9	9.8	16.1	5.1	8.2	17.8	21.6	46.5	38.4	0.3	1.2	1.3
16:1	8.5	6.4	4.9	38.6	39.6	0	20.3	17.7	22.0	60.7	65.9	68.0
17:0	0	1.9	6.2	8.6	11.1	13.5	0.4	0.4	0.4	7.6	5.1	6.9
17:1	8.6	0	0	20.8	21.9	0	1.3	1.4	1.2	2.4	0.5	0.5
18:0	0	0	0	0	0	0	0	0	0	0	0	0
18:1	0	0	0	0	0	0	0	0	0	0	0	0

[a] Expressed as a percentage of the total fatty acids

are replaced by C14, C16 and C17 saturated acids. *Vibrio* BM-2 shows a somewhat different response to decreased growth temperature in that increased proportions of shorter chain fatty acids (C8:0, C9:1, C12:1) are synthesized at 8°C and 0°C. The levels of hexadecenoic and heptadecenoic acids are relatively low at all temperatures compared with the other isolates studied and do not change significantly with temperature. In contrast, the principal component of *Vibrio* BM-4 is hexadecenoic acid which comprises some 60 % of the total free fatty acids acids at all growth temperatures. At 0°C significant quantities of heptadecenoic, pentadecenoic and tetradecenoic acids were also synthesized and unsaturated acids accounted for 76.8 % of the total. Raising the growth temperature to 8 and 15°C resulted in the loss of the C14, C15 unsaturated components although this was partially compensated for by the elevated levels of hexadecenoic acid at higher temperatures.

Effect of Temperature on Phospholipid Composition. Quantitative analyses of the extractable phospholipids of the four isolates are given in Table 3. The principal components are phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. In this respect the organisms have a similar composition to those described for other *Vibrio* spp. (De Voe and Oginsky, 1969; Oliver and Colwell, 1973). The two Antarctic isolates (*Vibrio* AF-1 and *Vibrio* AM-1), however, are atypical in that they contain significant quantities of phosphatidylserine at all growth temperatures. Phosphatidyl serine is the immediate precursor of phosphatidylethanolamine and for this reason it does not normally accumulate in significant quantities (Kanfer and Kennedy, 1964). *Vibrio* AF-1 additionally has three further phospholipid components. One has been identified as lysophosphatidylethanolamine since it has a low mobility in the solvents used and is molybdate and ninhydrin positive and cochromatographs with the authentic compound. The other 2 components have not as yet been identified but are ninhydrin and molybdate positive and periodate-Schiff negative. Acid hydrolysis of the two extracted components from preparative thin layer plates followed by analysis of the amino group using an amino acid analyser have so far proved inconclusive.

The most pronounced effect of temperature on the phospholipid composition of the 4 isolates is the marked increase in total phospholipids (expressed as total phospholipids/mg dry weight) as the growth temperature is lowered to 0°C. When determined on a percentage basis phosphatidylserine (PS), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG)

Table 3. Effect of growth temperature on the phospholipid composition of the 4 *Vibrio* spp. grown under glucose limitation. Figures in parentheses are percentage composition of each phospholipid

Phospholipid[a]	*Vibrio* AF-1 growth temperature				*Vibrio* AM-1 growth temperature			*Vibrio* BM-2 growth temperature			*Vibrio* BM-4 growth temperature		
	0°C	8°C	15°C	20°C	0°C	8°C	15°C	0°C	8°C	15°C	0°C	8°C	15°C
Phosphatidylserine	48.9 (27.1)	39.6 (24.7)	28.0 (20.5)	21.7 (21.7)	30.3 (17.5)	24.3 (17.3)	58.7 (43.8)	7.2 (4.9)	6.1 (5.2)	4.1 (2.9)	4.5 (2.6)	7.0 (5.3)	10.6 (8.9)
Phosphatidylglycerol	36.3 (20.2)	42.1 (26.2)	52.9 (38.6)	34.7 (34.7)	43.2 (24.9)	34.4 (24.5)	30.6 (22.6)	19.6 (13.4)	17.0 (14.6)	45.3 (33.8)	53.3 (31.5)	22.0 (16.7)	55.9 (47.0)
Phosphatidylethanolamine	26.2 (14.5)	24.5 (15.2)	18.1 (13.2)	17.1 (17.7)	24.6 (14.2)	33.1 (23.6)	16.3 (12.1)	67.8 (46.4)	46.4 (40.0)	53.5 (39.9)	25.6 (15.1)	21.2 (16.1)	13.3 (11.2)
Diphosphatidylglycerol	20.2 (11.2)	15.0 (9.3)	15.1 (11.0)	14.7 (14.7)	75.2 (43.4)	49.0 (35.0)	29.4 (21.2)	52.2 (35.7)	47.4 (40.1)	31.3 (23.3)	86.8 (51.5)	80.0 (61.6)	38.7 (32.7)
Lysophosphatidylethanolamine	11.5 (6.3)	7.4 (4.6)	6.7 (4.9)	2.1 (2.1)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Unknown A	7.2 (3.9)	4.3 (2.6)	4.4 (3.2)	1.7 (1.7)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Unknown B	29.9 (16.5)	27.4 (17.1)	11.9 (8.6)	7.6 (7.6)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total phospholipids	180.2	160.3	137.1	100.2	173.3	140.8	134.7	146.8	116.9	134.7	169.4	131.0	118.5

[a] μg phospholipid/mg dry weight
N.D. = Not detected

and lysophosphatidylethanolamine (lyso PE) show little significant change with temperature in *Vibrio* AF-1. Only PG shows a significant change with temperature in this organism. At 0°C PG accounts for 20.1% of the total phospholipids whilst at 20°C the level has increased to 34.7%. However, there is no decrease in DPG levels although there is a decline in the lyso PE concentration. Significant changes in phospholipid composition in *Vibrio* AM-1 do, however, occur. At 15°C, the isolates maximum temperature at which growth occurs, phosphatidylserine accumulates concurrent with a decline in PE levels. Diphosphatidylglycerol comprises some 43% of the extractable phospholipids at 0°C whereas at 15°C its synthesis is impaired and as a consequence the concentration decreases to 21% but there is no accumulation of its precursor PG. In sharp contrast to the Antarctic *Vibrio* spp. the PS content of the 2 North Sea isolates are low. In *Vibrio* BM-4 there is a steady increase in PS concentration as the growth temperature is increased. Concurrent with the increase in PS levels there is a decline in PE concentrations suggesting that PE decarboxylase (Kanfer and Kennedy, 1964) may be abnormally temperature sensitive. This is similar to that observed for *Vibrio* AM-1. Phosphatidylglycerol also accumulates in *Vibrios* BM-2 and BM-4 as the temperature is raised and this is accompanied by a decrease in DPG content in the phospholipid extracts suggesting that DPG synthetase (Short and White, 1962) is also temperature sensitive. From these data it would appear that at temperature approaching the maximum for growth in these isolates aberrations in phospholipid biosynthesis occur probably as a consequence of the inactivation of key enzymes such as phosphatidylserine decarboxylase and diphosphatidylglycerol synthetase.

Discussion

The data presented indicate that growth temperature exerts considerable effects on the fatty acid and phospholipid composition of the 4 psychrophilic *Vibrio* spp. Whilst the number of isolates studied is small the results demonstrate that decreasing temperature elicits different responses in fatty acid composition in each of the isolates. The 2 Antarctic isolates, *Vibrio* AF-1 and *Vibrio* AM-1 when grown at 0°C synthesize increased levels of unsaturated fatty acids, principally pentadecenoic, hexadecenoic and heptadecenoic acids at the expense of saturated acids. These data are similar to those reported by Kates and Hagen (1964) for *Vibrio psychroerythrus* and *Vibrio marinus* PS-207 (Oliver and Colwell, 1973). In contrast, *Vibrio* BM-2 maintains membrane mobility at low temperatures as a result of

chain shortening. This mechanism is similar to that reported by Russell (1971) in the psychrophile *Micrococcus cryophilus*. Whereas the isolates so far discussed respond to decreased temperature by synthesizing increased level of unsaturated fatty acids or chain length shortening, *Vibrio* BM-4 contains a high proportion of unsaturated fatty acids, notably hexadecenoic (60 %) at all growth temperatures. In addition, at 0° C additional levels of heptadecenoic, pentadecenoic and tetradecenoic acids were synthesized so that unsaturated fatty acids account for 76.8 % of the total. These unsaturated fatty acid levels are considerably greater than those reported for *Vibrio marinus* MP-1 (59.8 %) at 15° C (Olsen and Metcalf, 1968). Whilst these data show that major changes in fatty acid composition occur as the growth temperature is lowered the question still remains as to how the changes are expressed in the molecular architecture of the cytoplasmic membrane. The practical effect of the observed change in fatty acid composition is that sugar uptake in the *Vibrio* spp. proceeds at a maximal rate at 0° C (Fig. 1). These data are consistent with those reported for solute transport in a *Candida* sp. (Baxter and Gibbons, 1962; Lawrence et al., 1959) and a number of Gram-positive, psychotrophic bacteria (Wilkins et al., 1972) which are similarly cold resistant.

The phospholipid composition of the 4 *Vibrio* spp. are qualitatively similar to those reported for other *Vibrio* spp. (De Voe and Oginsky, 1969; Oliver and Colwell, 1973). The 2 Antarctic isolates (*Vibrio* AF-1 and *Vibrio* AM-1) contained significant quantities of phosphatidylserine, a phospholipid which does not normally accumulate as it is rapidly decarboxylated to give phosphatidylethanolamine (Kanfer and Kennedy, 1964). In contrast the 2 North Sea isolates contain small quantities of phosphatidylserine. Phosphatidic acid was also identified in lipid extracts from all the isolates, however, this component runs with the solvent front and as a consequence it was not possible to make any quantitative determinations. Antarctic isolate, *Vibrio* AF-1 has a considerably more complex phospholipid composition than any of the other isolates. In addition to the 4 phospholipids found in the other isolates 3 other unknown components were observed to be present. One of these has a low mobility in chloroform/methanol/H_2O (65:25:4 % v/v), is molybdate and ninhydrin positive and periodate-Schiff negative and has been identified as lysophosphatidylethanolamine. This phospholipid has been reported to be a widely distributed minor component of the phospholipids of marine *Vibrio's* (Oliver and Colwell, 1973). The 2 other unknown components in *Vibrio* AF-1 are also molybdate and ninhydrin positive and periodate-Schiff negative but so far have not been identified.

The principal effect of decreasing the growth temperature of the 4 isolates is to markedly increase the total phospholipid levels. These data are in agreement with the findings of Okuyuma (1969) who found that by lowering the growth temperature of *Escherichia coli* B from 37° C to 10° C resulted in a 20 % increase in total phospholipids. However, these results conflict with the data on the psychrotroph *Pseudomonas fluorescence* where no changes in phospholipid composition were observed on lowering the growth temperature (Cullen et al., 1971). On a quantitative basis the major changes in individual phospholipids involve PS, PG, PE and DPG. The effect of increasing growth temperature on PG levels in *Vibrio* AF-1 results in the accumulation of this phospholipids and a concurrent decrease in lyso PE. Similar changes have been reported to occur in *Vibrio marinus* PS-207 (Oliver and Colwell, 1973). In the North Sea isolates, *Vibrios* BM-2 and BM-4, growth at 15° C results in the accumulation of PG at the expense of DPG indicating that DPG synthetase is abnormally temperature sensitive. Similarly, in *Vibrio* BM-4, PS also accumulates at 15° C at the expense of PE suggesting that PE decarboxylase is also temperature sensitive. These findings of aberrations in phospholipid biosynthesis at temperatures approaching the maximum for growth of these isolates may help to explain the irreversible lysis which occurs in psychrophilic bacteria when exposed to temperatures 1 – 2° C in excess of the maximum for growth (Kates and Hagen, 1964; Kenis and Morita, 1968.

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